LATZER

A Bacteriological
Study of Milk

Agriculture
M. S.

1900

A BACTERIOLOGICAL STUDY OF MILK

...BY...

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THESIS

PRESENTED FOR THE DEGREE OF

MASTER OF SCIENCE

IN AGRICULTURE

IN THE UNIVERSITY OF ILLINOIS

JUNE, 1900

UNIVERSITY OF ILLINOIS

May 31 1900

THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

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ENTITLED A Bacteriological Study of Milk

IS APPROVED BY ME AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE

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A BACTERIOLOGICAL STUDY OF MILK.

The common phenomenon of souring of milk is due to minute organisms known as bacteria. However, all kinds of bacteria do not cause souring or coagulation of milk, certain of them affect it differently. Some forms, when present, produce pigment of different colors, some produce peculiar odors, while others grow in milk but produce no noticeable effect, and still others may live there but not multiply, The spoiling of meats, canned fruit, and in fact all putrefaction is usually caused by bacteria of some kind.

We must not, however, look at bacteria as altogether being detrimental to man, for they have economic values as well. We need bacteria to decompose our waste products. If we did not have them organic matter would pile up, and our soils would soon become exhausted, for the bacteria often figure greatly in the fertility of our soils. They are also made use of in commercial lines, e.g., in making vinegar, in ripening of cream, in making kumiss, etc, and the allied organisms known as yeasts are used in the manufacture of alcohol and bread.

Under natural conditions it is not often the case that one species brings about the desired result but generally there are different forms that enter the different steps. Consider for a

moment the proteids of milk; these are formed in their decomposition peptones, and in some cases ammonia. The peptones are often produced by a single kind but more often the secondary albumens are first formed, and from them by another transformation the peptones are produced. In the decomposition of sugars or alcohols, aldehides or keytones are first formed, and then acids are produced.

The work of bacteria can certainly be called a process of decomposition. In few instances are bacteria known to be able to build up material, from a lower to a higher form, The work of bacteria is therefore, and very properly, called a process of katabolism. That is they have usually no power to utilize the sun's energy. In this respect they differ from the higher plants and they must rely altogether upon the media in which they live as their source of energy. The exceptions are only in regard to a few species living in soil. These seem to utilize energy in the form of heat and thus may live on inorganic matter and build up complex from simple chemical substances. One or more species of bacteria also have a purple colored substance by which they have like green plants, the power of decomposing carbon dioxide when exposed to light.

Bacteria are the smallest living organisms: they are single celled and belong to the lowest form of plants. These organisms are so minute that they can only be seen with the aid of high magnifying powers, and some, e.g. those present in the vaccine for small-pox, are so small that they can not be seen even with the best microscope and the best methods of staining.

Bacteria differ somewhat in form and size, but the difference is so small, that in most cases they can not be separated into

into species, by the use of the microscope alone. In regard to form they are divided into three main divisions, viz.- 1. Coccus, 2. Bacillus, and 3. Spirillum. All bacteria may be referred to one of these three divisions, but there may be gradations from the types.

When bacteria are globular and single they are called Micrococcus, but if they are in pairs they are called Diplococcus. Sometimes they are attached and form long chains, they are then called Streptococcus. If they form clusters instead of chains they are called Staphylococcus. The term Bacillus includes all rod shaped forms. These vary greatly in length and width, often in the same species, especially when grown under different conditions. The term Spirillum includes all forms that are spiral or that have a tendency to be so, e. g. curved forms are classed here as well as those that have a corkscrew shape.

The distribution of bacteria is very wide, for they are found in air, soil, water and dust; on our clothes, on our skin, in our food, and the alimentary canal,- in short in almost every conceivable place. This is not so because they have great power of locomotion, for they have no motion, except very slight wiggling motion when in a liquid medium, but it is true because they are so very small and light that they are easily carried about by currents of air, especially on dust particles, however minute they may be.

Bacteria can not often live for any great length of time, suspended in the air, because the conditions are such as are detrimental to their life. This can not be said of the forms that are found in the soil, for the conditions there as to moisture, temperature and light are favorable for the kinds found there.

Most of them, however, grow best within a few inches of the surface, but some may be found at a depth of several feet.

Most waters, especially sewage add drainage waters, contain sufficient organic matter to serve as food for bacteria. Bacteria may fall into water from the air or be washed out of the soil, but many forms are present which are not usually found in the air or soil, especially is this true of stagnant pools rich in organic matter. Well water, especially of deep wells is as a rule, quite free from bacteria, for the thick layer of soil through which the water passes to reach them acts as a filter.

The growth of bacteria like all other living organisms bear a direct relation to their surroundings. Certain conditions are absolutely necessary before they can develop. Other conditions, though of advantage, are not of such vital importance. The three most important conditions are known to be; (1) food supply, (2) temperature, (3) moisture.

Regarding the food products, much difference exists for different kinds. Most of the disease producing forms are very particular in their ~~in their~~ selection of foods. The more common species, or such as live in milk, are not so delicate in this respect. Their food supply can best be known from the places in which they naturally abound.

Food for bacteria in order to be available must be in solution, for it must pass through the cell wall by osmosis. They do sometimes live on solid material, but in such cases they are able to produce enough chemical change by bringing material into solution to support themselves. The food must contain nitrogen, carbon,

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oxygen and small quantities of mineral matter. As a rule the first two are best made use of when in organic form rather than when in the inorganic condition. Milk contains all these substances and in a form that can readily be utilized. We find for this reason that a large number of kinds will grow in milk. If media are too concentrated bacteria can not grow in them hence jellies or syrups are not suitable for their use. After sufficient dilution of such materials, the bacteria grow very readily in them.

Regarding the degree of heat necessary for the growth of bacteria it is similar to that of other plants, a certain degree of warmth is necessary for their activity. The temperature at which bacteria grow best varies with different species. The forms living within our bodies, that is the disease producers, grow best at the temperature of our bodies. These forms do not grow except within a narrow range of temperature, but most of the other forms are not so particular. There are, however few forms that multiply below 4 degrees ~~and~~ but there are some known that can live and multiply at zero Centigrade. When the temperature goes below this they fall into a torpor from which they awaken only after the increase in temperature. When they are in such torpor-like condition they can resist a great amount of cold.

It is, however, not only cold but also heat which arrests the development of bacteria. Few forms are able to grow above 50 degrees C., some have been found to live and multiply at from 65 to 70 degrees. At higher temperatures they are quite easily killed, except when in the spore state. A few trials gave me the following results:

Bacteria	at 48 C. 1/2 hr.	at 50 C. 1/2 hr.	at boiling 1/2 hr.	at boiling 20 min.	at boiling 15 min.	at boiling 10 min.
Bacillus.						
NO. I.	GREW	GREW	----------	----------	----------	----------
NO. II.	GREW	----------	----------	----------	----------	----------
NO. III.	GREW	----------	----------	----------	----------	----------
NO. IV.	GREW	----------	----------	----------	----------	----------
NO. V.	GREW	----------	----------	----------	----------	----------
NO. VI.	GREW	----------	----------	----------	----------	----------

The descriptions of the kinds tried is given later. The aim was to have new cultures free from spores.

It was mentioned above that the food of bacteria must be in the liquid condition. A certain amount of moisture is therefore necessary, before growth can take place. Or ganic substances such as fresh animal and vegetable tissues contain sufficient water to permit growth.

A large number of bacteria require the presence of oxygen, and would die if they had not access to it. These forms are called AEROBIC bacteria. Oxygen, on the other hand is detrimental to many forms; these are known as ANAEROBIC forms. Many thrive both in the presence and absence of oxygen; these are sometimes said to be areobic and facultative anaerobic.

The great majority of bacteria prefer darkness, but some will grow in direct light. Direct sunlight as a rule kills bacteria. It is therefore the cheapest way to kill these organisms. This is of the greatest advantage to the majority of dairies, especially in the smaller ones where steam is not accessible. It seems to keep bacteria in check, for the utensils, when thus exposed are

practically sterile.

Bacteria multiply very rapidly if they are not checked in some way, for under favorable conditions the more rapidly growing kinds multiply once every thirty minutes. They reproduce by division. A single cell divides, and gives rise to two cells, each of these again divides and so on. Now if there were no check and they continued to double themselves every thirty minutes they would soon fill the seas and oceans. This is, however, not the way they actually do in nature, for the conditions are not always perfect. The food supply, the conditions of temperature and moisture, are not always ideal for the growth of these organisms, but very often such as will kill them in great numbers. There is another way by which bacteria reproduce themselves, but it is not a method of multiplication. When conditions are not favorable for these organisms, they have the power to produce what are generally known as SPORES. The cell contents gathers up into a small lump and a thick wall forms over it, similar to a seed in many respects. In this spore condition they are able to endure many hardships. They withstand boiling water for a considerable time. They are not killed for want of food or for lack of moisture. When conditions again become favorable these spores germinate and form a new cell which has the power of division the same as above described.

Many kinds of bacteria are useful in dairying, as well as in other arts. Ripening cream is probably the most important process in which bacteria are made use of in our more common dairies. They are however, very essential in the ripening of cheese. In the former case they put the cream into better condition for

churning and imparts to butter a much better flavor. So well is this fact established that within recent years there have been companies formed to deliver to us cultures which will produce better butter. These will, however, become harmful when the milk should be preserved. So also when we consider the putrefactive forms in the right light, they are useful. They may not be useful in the art of dairying, but in other lines, e. g. the putrefying forms causing decomposition of organic matter thus producing humus in the soil.

The bacteria so far mentioned do not cause disearse and are therefore known as NON-PATHOGENIC to distinguish them from the PATHOGENIC or disease-producing forms. The non-pathogenic forms do not cause any great derangement when taken into the body, even when they gain entrance in large numbers. The great majority of bacteria belong to this class.

The pathogenic bacteria cause some form of derangement or other. The body is able to resist the attack of many of these germs especially when it is in good health. When, however, a person's body is not in good condition these forms very readily do him injury. That is when a person is not feeling well he is more apt to contract a disease than when he is in a healthy vigorous state:

Most of the pathogenic kinds produce a toxin or poison which is the direct cause of the evil and causes the fatal results. All diseases that are contagious are produced by bacteria or similar living things and many that are not contagious in the common sense of the term, e.g. tetaneous or consumption. The diseases of our domestic animals are caused by bacteria, as well as those of man

and in many cases the same species will produce similar effects in man and beast. Some forms will only grow on one species of animal but closely allied forms may develop on different species.

The way bacteria spread from one patient to another is not always sufficiently plain for us to follow the process. But some of the most common ways are by the wind, on our clothes, in the drinking water and in our food. Milk is a very good conveyor of them, either by carrying the disease from animal to man or by the milk getting contaminated and thus communicating the germs to man. From milk, butter and cheese are often infected, but it is claimed by some eminent scientists that butter and cheese are detrimental to the life of pathogenic bacteria, at least to certain species.

In milk,bacteria multiply rapidly, but they are quite readily exterminated by sufficient increase of heat. Some of the more common kinds in milk are named below. After each species is placed the temperature at which they are killed by ten minute's exposure. These figures are from Sternhug's MANUAL of BACTERIOLOGY. Bacillus of tuberculosis 75 C.; Bacillus of diphtheria, 58 C.; Bacillus of glanders, 55 C.; Bacillus of typhoid fever, 56 C.; Diplococcus of pneumonia, 52 C.; Spirillum of Asiatic cholera, 52 C.; Bacillus Coli-Communis, 68 C.

We see from this that milk is freed from all dangerous germs at a temperature considerable below boiling but if not so subjected to heat they will live in milk for an indefinite length of time. In butter according to H. Laser, vol. III, E.S.R. p. 422, pathogenic bacteria live only 5 or 6 days. H.Weigmann, vol. VI, E.S.R. p. 168, states that cholera bacteria actually multiply in milk,

but when the milk is employed in cheese making they died nine hours after the addition of rennet. He concludes that there is, therefore, no danger of infection from cheese.

The one disease which is probably most often spread through milk is tuberculosis. The greatest danger of the presence of this germ in milk is when the cow has tuberculois of the udder. Milk can not very readily be tested for these germs, but the most practical method is that given by W Thörner. "Milk is first treated with a potash solution to saponify the fat, then with acetic acid to dissolve the casein. After this the whole is whirled in a centrifuge for ten minutes. The liquid is then poured off, the sediment washed with water and examined under the microscope after staining in the usual manner".

Milk as it exists in the udder of a healthy cow is usually sterile, but bacteria gain entrance into the teats and often get up a considerable distance and after they have entered they multiply rapidly because the conditions for their growth are ideal. It follows from this that the fore-milk or the milk first drawn is apt to be very rich in bacteria and for that reason furnishes a large supply of bacteria to the whole milk. The stable air is another source from which the milk receives a large supply of its bacteria, and especially is this true if feeding, handling of bedding, or anything else that will raise dust, is going on during milking. The air of the barn is nearly always well supplied with bacteria, but the raising of great clouds of dust through which the milk gets more than its necessary share of germs can easily be prevented by not stirring feed or litter within an hour or two previous to milking.

Small particles of dust or dung sticking to the hair of the cow are easily rubbed off and may fall into the milk. This, especially in the case of the latter substance can be prevented, partially, by supplying good bedding. The small particles lodged among the hair, generally small scales of skin are very rich in bacteria. These gain access into the milk unless the cows are kept exceptionally clean. The contamination from this source can be greatly reduced by dampening the udder and flanks just previous to milking.

Another and quite extensive sort of infection of milk is from the clothes and hands of the milker. Our hands are not germ free when clean and very far from it, after handling dusty feeds etc. The milking should in no case, be done with wet hands-- a custom so common that we even, sometimes, hear it asserted that it is impossible to milk with dry hands. This is, however, not only possible but absolutely necessary. The clothes of the milker supply a great many bacteria. The milker should have an extra suit to milk in, and this should be, preferably, white so as to show all dirt. These clothes can be light and large enough so that they easily slip over the other clothes.

All utensils which are used in handling milk ought to be sterile. In our modern dairies where steam is available they are easily rendered so, but in most dairies where steam is not used the next best thing is to expose them to direct sunlight for several hours. The utensils, especially the buckets, should be made of tin and not wood, and these tin vessels should be as free of seams as possible, for here is where the milk lodges and is hard to clean out. The hard dry particles of milk lodged in these crevices are literally loaded

with bacteria and will contribute their share to the milk.

After the milk comes from the barn it is necessary to cool it quickly for these bacteria multiply very rapidly. Milk when it is drawn from cows is very near the optimum growing temperature, for many bacteria, and it is therefore necessary to change these conditions as soon as possible. If it is cooled quickly the growth is checked and many of the spores will not germinate. It is also necessary to remove milk from the barn as soon as possible after drawing to prevent any further contamination and also to prevent the absorption of odors. Milk is a good medium for bacteria to grow in and they will multiply very rapidly. If conditions of temperature are favorable milk spoils within a few hours. As a rule one form predominates but by no means always the same species. The species that predominate generally produce a by-product which is detrimental to the other forms and tends also to check the growth of its own species. This is, however, of no commercial value to us, for milk, in order to be marketable, must be as sweet as possible. The simplest but not the most efficient method in keeping milk sweet is to place it in as cold a place as possible.

Milk left at ordinary room temperature or about 20°C. will sour in from twelve to twenty-four hours. When the temperature is lowered to about twelve degrees C. the period is much longer and several trials gave me the following results: several samples of milk were sterilized. One of these was inoculated with *B. acidi lactici*, one with *B. butyricus*, and another with a mixture of the above two. These were kept by me in running tap water at about 12°C. and the milk soured within about two weeks. The milk inoculated with *B. butyricus* spoiled first, then the mixed sample and lastly the one with *B. acidi lactici*. This seems to show that *B. butyricus* grows more rapidly than *B. acidi lactici* at low temperatures.

Another set similar to this was kept in the University dairy ice chest at a temperature of two to three degrees C. After two

months the milk was still sweet and it was then tested for the number of bacteria present, and it proved to be practically sterile. This tends to show that milk kept at this temperature is not soured by these forms. This milk was, however, subsequently found to be spoiled, not by the species with which it was inoculated but by B. Fluorescens Putidus. The milk probably got infected when the above mentioned test was made.

The rapidity of growth at higher temperatures was also tried, by planting certain cultures in agar plates, then subjecting these to different temperatures and noting the size of the colonies. For this purpose Bacillus No. I, No. II, No. III, and No. IV, described later on were used. A plate of each of these was exposed at room temperature or about twenty degrees C., one in an incubator at about thirty-three degrees C, and another in an incubator at about thirty-seven degrees C. In every case the one left at room temperature grew least, at the other temperatures they were practically the same. The first two having slightly larger colonies at thirty-three degrees while the latter were in favor of thirty-seven degrees. This method of procedure is only comparative and far from accurate.

Another method was tried in determining the growth of B. acidi lactici. This was by testing the amount of acid produced in a certain time. One culture of B. acidi lactici was obtained from sour milk and the other was received from the Duplex Butter Culture Co. One-twentieth normal sodium hydroxid was used in titrating to determine the amount of acid. The first mentioned sample produced acid a little faster, and it was also noticed that the latter, after several transfers in bouillon would not coagulate

milk. This method can not be used in testing the rapidity of growth between different species, for some produce acid more rapidly and in greater quantities than others.

It at once appears very evident that the number of bacteria in milk when it reaches the consumer, depends on how the milk is treated when in the barn, how it is cooled and how cool it is kept subsequently. The dairyman can not control all these conditions except in extraordinary circumstances. In most of our dairies the seasons make a great deal of difference. What would be expected is to have more contamination in winter than in summer, because the cows, as a rule,are dirty and are kept in the barn. There is a great deal of handling of litter, hay, etc., and this causes a great amount of dust to float around in the air. The barn is seldom well aired for the dairyman is afraid to get the barn too cold for his animals. To counterbalance this is the cold weather and it is easy to cool milk and to keep it cold, thus greatly checking growth. In summer the animals are clean, for as a rule they run in the pasture and only lie down where there is a good clean place. Nothing is handled in the barn to raise dust, but the conditions of temperature are first class for the development of bacteria. It is a tedious job to cool milk properly and just as hard work to keep it cool. To see whether the number of bacteria varies with the seasons three collections were made from each of the five dairies. The first was made in winter when it was very cold; the next when it was quite warm but before the cows were turned on pasture, and before they were left out of doors at night; and the last was intended to be late enough so that the cows were

on the pastures and left out doors at night and was made about the middle of May.

The way the bacteria were counted was as follows: 1 c.c. of milk was diluted with 100 c.c. of sterile water. With another sterile pipette 1 c.c. was added to a melted agar tube and the entire contents poured into a sterile plate. If the number of bacteria is great they are too many to be counted, so 1 c.c. of the above 100 c.c. was taken and diluted with another 100 c.c. of sterile water, thus making a dilution of 1 to 1/10,000. of this 1 c.c. was plated as before mentioned. These two dilutions used, because in most cases there was no method by which to estimate the number, and the wide variation was overcome by making this great allowance in plating. When the number was small the first dilution was used in counting but if it was large the other was used. The number obtained in 1 c.c. of each of the samples collected was as follows:

Name of Dairy	First collection	Second collection	Third collection
U. of I.	22,700	18,000	11,000
Dobbins	15,000	55,000	617,400
Haines	230,000	118,800	182,000
Corray	285,000	114,000	40,000
Graham	504,000	59,000	1,020,000

These samples were plated as soon as they reached the laboratory. They never were plated at the same time so these figures represent the milk at the time it was delivered. The U. of I. milk was received about eight o'clock every morning, Dobbins', Corray and Graham delivered theirs soon after eight. Haines was not received in the laboratory before one o'clock. It was collected at a private house at about ten or eleven o'clock and then was kept on ice until after dinner. The University of Illinois and Corray milk was well cooled while that of Dobbins and Graham was not. Dobbins' first delivery was apparently not cooled at all, and was probably delivered immediately after milking.

From the facts known in this experiment that on proper cooling we can get purer milk in summer than in winter, that is, the contamination is less then than in winter. On the other hand if the milk is improperly cooled and deliveredwhile it is yet warm the number of bacteria is much larger in summer than in winter. It is evident that the purity of the milk or the number of bacteria in the milk does not so much depend upon the season as upon how it is handled, before it reaches the consumer.

From the figures in this experiment we also get some idea of the number of bacteria in milk as it is generally delivered. In the above it varies from 11,000 to 1,020,000 per c.c. At Madison, Wisconsin, it was found that milk ranged from 15,000 to 2,000,000 per cubic centimeter. We should expect much purer milk in small country towns where the milk is delivered the same day it is drawn and sometimes twice a day, that is immediatly after each milking. In large cities where the milk is shipped in by rail and often great distances it usually takes from twenty-four to thirty-six

hours from time of milking until it reaches the consumer. In such cases the milk is often very near souring and the number of bacteria is very great. A sample of milk almost sour was found to contain, by actual count, about 19,000,000 bacteria per cubic centimeter. Another, showing no signs of souring contained 9,500,000 per cubic centimeter.

Milk is often unfit for use on account of some peculiar odor which it possesses. This odor may be due to several different causes. One very common way that milk receives odor is by the cow eating some food which possesses a strong and peculiar odor and this is often transmitted to all the secretions of the body, but to milk especially, materials that cause such odors in milk when eaten by the cow are: onions, turnips, rape, distillery slops, etc. The only preventative or remedy for such tainted milk is not to let the cows have any of these materials that will transmit the odor to the milk. Milk will often become tainted by direct physical absorption of odors from the barn or dairy. At the Wisconsin Station it was found that both cooled and warm milk will absorb odors, the latter however much more readily.

The most common and the most numerous cases of tainted milk and milk products are caused by bacteria. Almost every species of bacteria produces an odor or flavor of its own, and these will vary from agreeable to very disagreeable ones. If the odor is due to bacteria and appears from day to day, the infection is from the same source and in such a case it is well worth looking for. In many creameries, such cases have been traced to bad floors or wooden platforms. In such places milk is spilled, and it can not be thoroughly cleaned. These places as a rule remain damp all the

time thus affording excellent growing conditions for bacteria.

We have seen that the number of bacteria, and also the damage caused by them can be greatly reduced by cleanliness, and by keeping the milk at as low a temperature as possible. By no practical method is it possible to produce absolutely sterile milk, because the sources of contamination are so numerous, it is almost impossible to eliminate them all. The only way to get sterile milk on a commercial scale is by killing those bacteria present. This can be done by adding poison but this would render the milk worse than useless for consumption and for almost every other purpose. It might do well enough to preserve milk for future analysis. We must therefore resort to other methods. Heating milk to the boiling point will kill all those in the vegetative state but the spores would not certainly be affected. These would soon develop and the boiling would be for naught except possibly for extending the time during which it will keep sweet.

Our pioneer bacteriologist soon found that by killing the bacteria in a vegetative condition, then after setting the medium away for the spores to develop, by heating again, sterilization can be effected. The method most commonly practiced is to heat this medium to the boiling point and so keeping it for about fifteen minutes on each of three successive days. Milk is often rendered sterile in this way in the laboratory where it is to be used for experimental purposes.

In the processes of condensing milk to a small bulk, it is of the greatest importance to render this milk sterile or the work would be of no avail; for this condensed milk is often shipped great distances and it may be years before it is consumed. There

need be no danger in the consumption of such sterilized condensed milk because through it no disease will be communicated.

To render milk absolutely sterile requires very expensive machinery and also a great deal of labor. It is however not necessary to heat it as often as above mentioned when heated under pressure; but it is still a very expensive method to render milk sterile and it does not pay if the milk is intended for immediate consumption. This difficalty of cost is in some measure overcome by the process of pasteurization.

The object of pasteurization is to kill all the germs in the vegetative state. Pasteurization is as a rule done at from 60° to 65°C. This is high enough to kill most bacteria and at this temperature milk will not acquire a cooked taste which is so much disliked by many people. By heating the milk at the above temperature from twenty to thirty minutes most of the bacteria are killed except those in the spores condition. The more common forms of disease germs spread through milk (tuberculosisb typhoid fever, etc.) do not produce spores, so pasteurization greatly eliminates the danger of spread of these diseases through milk. The keeping quality is also greatly improved by killing the greater part of the bacteria present in the milk. It will keep sweet several days longer than would be possible without it, for the keeping quality of milk greatly depends upon the number and kinds of bacteria present in the milk.

To test the efficiency of pasteurization, between the number of bacteria present in milk and also the efficiency of quick and slow heating and cooling, several experiments were tried. Pasteurization was done by heating about 125 c.c. of milk in a 250 c.c. Erlenmyer flask.

These flasks were placed in a sterilizer containing water at 65°C. It was intended that the milk should be at this temperature for twenty minutes. The experiment was as follows. Flasks I and II were heated quickly, that is they were immersed in water at 65 degrees; it was found by previous experiments that it took about six minutes for the milk to get up to the same temperature as the water. After the temperature was up for twenty minutes they were cooled as quickly as possible in cold water by constant shaking. It took only a little more than a minute to so cool the milk. Flasks III and IV were heated up slowly, that is by placing them in cold water and heating the water. It took about fifteen minutes to heat up the water to 65 degrees and about three minutes more until the milk was at that temperature. These after being at 65 degrees for twenty minutes were set out into the room to cool down slowly. Flasks V and VI were heated up quickly and cooled slowly as has already been described. Flasks VII and VIII were heated slowly and cooled quickly. The odd-numbered flasks were just ordinary milk from the dairy. The milk in the even-numbered flasks was previously inoculated by a broth culture of *B. acidi lactici*, ~~and~~ *B subtilis*, *B. mesentericus vulgatus* and *B. butyricus*. It was the aim to have these cultures old so that they contained many spores. The number of bacteria was determined in the samples before pastureization, and then again between two and three days after it. The milk in the meantime was kept in a rather cool place. It was not so much the exact number of bacteria present but the relative number present after these treatments that was looked for. The figures thus obtained are given in the following tables.

PASTEURIZATION EXPERIMENT.

	Original Milk		Inocculated Milk
Before heating			
Exp. No. 1.	20,500	Exp. No. 1.	1,462,000
" " 2.	17,500	" " 2.	1,344,000
" " 3.	18,000	" " 3.	864,000
" " 4.	7,700	" " 4.	1,304,000
" " 5.	10,000	" " 5.	470,000
" " 6.	42,000	" " 6.	2,551,000
Heated quickly and cooled quickly.			
Exp. No. 1.	160,000	Exp. No. 1.	440,000
" " 2.	500,000	" " 2.	1,267,000
" " 3.	32,000	" " 3.	3,456,000
" " 4.	81,900	" " 4.	510,000
" " 5.	115,000	" " 5.	170,000
" " 6.	740,000	" " 6.	1,227,000
Heated slowly and cooled slowly.			
Exp. No. 1.	3,190,000	Exp. No. 1.	4,300,000
" " 2.	1,700,000	" " 2.	2,400,000
" " 3.	1,400,000	" " 3.	18,700,000
" " 4.	347,400	" " 4.	1,020,000
" " 5.	180,000	" " 5.	1,200,000
" " 6.	2,100,000	" " 6.	5,300,000
Heated quickly and cooled slowly.			
Exp. No. 1.	900,000	Exp. No. 1.	1,650,000
" " 2.	1,780,000	" " 2.	1,800,000
" " 3.	800,000	" " 3.	7,360,000
" " 4.	327,000	" " 4.	1,360,000
" " 5.	170,000	" " 5.	210,000
" " 6.	1,400,000	" " 6.	5,300,000
Heated slowly and cooled quickly.			
Exp. No. 1	1,160,000	Exp. No. 1.	1,830,000
" " 2.	2,100,000	" " 2.	2,100,000
" " 3.	———	" " 3.	3,900,000
" " 4.	195,300	" " 4.	813,800
" " 5.	150,000	" " 5.	190,000
" " 6.	850,000	" " 6.	2,100,000

In every case the inoculated milk had a great many more bacteria after pasteurization as well as before. The difference however is not as great after heating as it was before. Heating quickly and cooling quickly has in every case the least number of bacteria. Heating slowly and cooling slowly in every case has the highest except in the second experiment, with the original milk, in heating quickly and cooling slowly and heating slowly and cooling quickly; also in the fourth experiment in the inoculated milk, in heating quickly and cooling slowly. The result of heating quickly and cooling slowly is higher than the heating quickly and cooling quickly but lower as a rule than heating slowly and cooling slowly. In the first two experiments in both the original and inoculated is it lower than the heating slowly and cooling quickly. In the other four experiments heating slowly and cooling quickly gave better results.

We may conclude from these few experiments that: 1. the cleaner or the freer the milk from bacteria, the more effective the pasteurization. 2. Heating quickly and cooling quickly is the most effective method of pasteurization. 3. Heating quickly and cooling slowly and heating slowly and croling quickly are more effective than heating slowly and cooling slowly. 4. Heating slowly and cooling quickly is more effective than heating quickly and cooling slowly. If by some method the temperature could be raised quicker, heating quickly might be more effective than it now is as compared with cooling quickly.

It is universally understood that bacteria as a rule can not be distinguished and classified by their appearance, but their characteristic growth on different media is absolutely essential

for this purpose. Because we can not watch the growth of one special bacterium and since we must resort to the watching of groups or colonies, it is necessary that we have only one species present or what is usually known as a PURE CULTURE. As bacteria are found in nature they are nearly always mixed and it is therefore necessary to make separations so that the growth of a single bacterium is obtained. The best and most ready way to make separations is to grow them on solid media such as agar or gelatin. The medium is first melted, then it is inoculated with the material containing the form wished to be separated. The entire contents of a tube is then poured into a Petri dish where it is allowed to grow until all the colonies plainly show. Each colony represents the growth from a single bacterium and a pure culture can easily be obtained by inoculation into other media from one of the colonies.

The media used in this work for the determination of species were: bouillon, gelatin, agar, potato, and milk; litmus milk was used to test for acid formation. Bacteria like all other plants grow differently in different soils; it is therefore necessary that the media should each be alike every time and always be made according to a definite formula. The formula used for making media for this work were the same as th~~ose~~at used by Fuller and Copeland in their water investigations, a full account of which may be found in Massachusetts State Board of Health Report, 1895. Briefly the processes are as follows:

Bouillon, beef infusion, is made by placing one pound of finely chopped meat, as free from fat as possible in one liter of water. It is set away in a cold place, preferably in an ice-chest, for twenty-four hours, while still cold it is strained through double

thickness of cheese-cloth in order to remove all fat and particles of beef. One percent of peptone, that is ten grains to the above amount is added while boiling. Now boil for about ten minutes and then strain out the precipitated particles and make up the solution exactly to one liter. Next it is made strictly neutral to phenolphthalein. After it is thus neutralized it is made acid to the desired degree, by adding normal hydrochloric acid. For my work ten on Smith scale was used, that is, ten c.c. of normal acid to the liter. This was used because it corresponds quite closely to the acidity of normal milk and it is what is most commonly used. The bouillon was then boiled for a short time, filtered and sterilized, After the first batch all of this medium was made by using Leibic's extract of beef, five grains to the liter of water, instead of the beef infusion. When the extract is used the white of an egg must be added to clear the solution.

Nutrient gelatin is made by adding to the water containing five grains of the extract one per cent of peptone and twelve and one-half per cent of gelatin. Heat the broth until the gelatin is dissolved. Then cool neutralize and add the whites of two eggs. Boil over a free flame until the liquid becomes clean, that is about ten or fifteen minutes. Now fill up to one liter then neutralize as before and add the required amount of acid. Again boil for about two minutes, then filter, fill up to exactly one liter and lastly sterilize.

Nutrient agar is made by adding to the solution of extract and peptone one and one-half percent of agar-agar. The agar should previously be dissolved in about three-fourths of a liter of water. The mixture is then boiled over the free flame until only about

one liter remains. Next it must be cooled and the whites of two eggs added. It is again boiled over the free flame until the coagulum is well separated and the liquid clean. Now make up to one liter neutralize and acidulate. Boil it for a minute or two, filter and sterilize.

The growth on potato was done in tubes and not on whole potatoes. Regular potato tubes were taken, that is those with constrictions near the bottom. In some cases regular large size test tubes were used in which were placed lumps of cotton to support the potato, to keep it out of the liquid which exudes from the potato on boiling. The potatoes were first well washed to get them as free from bacteria as possible. Next they were peeled and sections cut with an ordinary apple corer. These cylindrical sections were cut tapering at one end and inserted that end up into the tube. The tube was then plugged and sterilized. At first they were sterilized by the three day method, heating fully for one-half an hour on each day. No difference in growth could be seen when sterilized in this way or by the use of the autoclave, so in the latter part of this work the potato tubes were sterilized in the autoclave at ten pounds pressure for one half hour.

For milk preparations fresh skim milk was used. It was sterilized in the autoclave at ten pounds pressure. The litmus tests were made by adding to milk enough litmus to give it a slight blue color. It was sterilized the same way as was the other milk.

The process of staining is so simple that it need not be mentioned here with the exception of staining of B. <u>tuberculosis</u> which is very hard to stain. These bacteria are very slow in taking color and just as slow in giving up their color when acted upon

by decolorizing agents. This furnishes a very efficient method of detecting tuberculosis. Its detection in milk is possible as mentioned above by the following method, recommended by W.Thorner reported in the Exp. Sta. Record, vol. V p. 1045 . Treat the milk with a potash solution to saponify the fat, and acetic acid to dissolve the casein. It is then whirled in a centrifuge for ten minutes. The liquid is then poured off, the sediment washed with water by whirling, and the residue examined under the microscope, after staining on the cover glass. A very good way of obtaining and one that is often used is Ziehl's method. The material for examination is fixed on the cover glass. To stain it is floated film side down in carbolic fuchsin, which is prepared by adding to ten c.c. of saturated aqueous solution of carbolic acid eleven c.c. of saturated alcoholic solution of fuchsin. The time required to stain is about a half hour or about two minutes when the stain is boiling hot. The excess of dye is washed off with water and the specimen decolorized in five to ten per cent nitric acid. When the color begins to disappear it is at once transferred to sixty or seventy per cent alcohol where it is left until almost decolored. Wash the cover and examine. It is often of advantage to stain slightly with methylene blue, before examining to give contrasts.

Next will be enumerated the species studied in this work. The different forms were collected from different sources but principally from spoiled milk. Some forms obtained from other sources for the express purpose of seeing if they affected milk. First will be given the species whose names I have been able to find in Stenburg's Manual of Bacteriology and Eisenberg's Bakter-colische Diagnostic. Then will be given the bacilli not determined. Then the Micrococci and Diplococci.

BACILLUS MESENTERICUS VULGATUS.

HABITAT. In milk, it may also be found in soil, water, etc.

FORM. Short thick rods with rounded ends, rather small ~~ends,~~ sometimes in pairs.

MOTILITY. Actively motile.

OXYGEN REQ. Aerobic, grows also to the bottom of the stab.

GELATIN. Small white colonies spreading and liquefying, later forming a pellicle over the liquefied portions.

GELATIN STAB. Growth occurs along the entire line, liquefaction most rapid near the surface; liquefied portion is extended through the entire tube, and has a white pellicle covering it.

STREAK CULTURE. On AGAR a grayish white thick growth, gradually becoming wrinkled or folded. On POTATO it grows especially well covering the entire surface with a thick folded growth.

BOUILLON. Growth produces turbidity, with a granular, friable, pellicle over the surface.

MILK. Casein is coagulated and then almost completely peptonized, the remaining coagulum settles to the bottom and the liquid is slightly yellowish.

ODOR. Has a peculiar cheesy odor.

Litmus. Produces acid.

SPORES. Large oval spores are formed, very readily, located at one end of the rod.

STAIN. Redily stained with gentian violet.

TEMPERATURE. Grows redily at room temperature, also at 37 C.

REMARKS. It is one of the species commonly called "Potato Bacillus."

BACILLUS CYANOGENUS.

HABITAT. Found in milk, also in sewage water.

FORM. Short rods with rounded ends about three times as long as wide. It is often found in pairs.

MOTILITY. Quick darting motion.

OXYGEN REQ. Aerobic, grows also to the bottom of the stab.

GELATIN. Small round colonies which under the microscope are yellowish and granular.

GELATIN STAB. An abundant growth along the entire line of inoculation, but no liquefaction.

STREAK CULTURES. On AGAR a rather dark, gray colored moist growth, the dark color penetrating the medium. On POTATO slight amount of white colored growth, apparently moist on the surface.

BOUILLON. Grows rather slow, forming a brown colored liquid and precipitate.

MILK. Not coagulated but seems peptonized and assumes a peculiar slate gray color.

ODOR. Peculiar putrefactive odor.

LITMUS. No acid is produced.

SPORES. Not observed.

STAINS. Quite readily.

TEMPERATURE. Grows best at room temperature, fails entirely at 37 C.

REMARKS. Often called bacillus of blue milk. Produces a blue color when acid is present.

BACILLUS VIOLACEUS.

HABITAT. Sewage water and milk.

MORPHOLOGY. Long slender, rather large rods.

MOTILITY. Quite actively motile.

OXYGEN REQ. Aerobic, grows also to the bottom of the stab.

GELATIN. Grows quite well, forming small, white, irregular dots, gradually liquefying and turning blue.

GELATIN STAB. Growth along the entire line of inoculation, liquefying most rapidly from top.

STREAK CULTURE. On AGAR it grows well, forming a thick, moist, violet colored film, the color penetrating the medium. On POTATO a small amount of very deep, violet colored, rather dry growth.

BOUILLON. Forms a violet growth in the medium and like a vise a violet covering over it.

MILK. Coagulated rather slowly and a distinct violet color appears after three or four days.

ODOR. Very peculiar, musty and putrefying.

LITMUS. No reaction.

SPORES. Not observed.

STAINS. Reacts readily with methylene blue.

TEMPERATURE. Grows best at room temperature, fails entirely at 37 C.

BACILLUS COLI COMMUNIS.

HABITAT. In the intestines of man and beasts.

FORM. Rather small short rods, about two times as long as wide.

MOTILITY. Sometimes slowly motile, often without motion.

OXYGEN REG. Aerobic, grows also to the bottom of the stab.

GELATIN. Small white spreading colonies, peculiarly spreading from the center with fringed edges , moist appearance, yellowish granular in structure under the microscope.

GELATIN STAB. White growth all along the line of inoculation and over the surface.

STREAK CULTURES. On AGAR a moist white growth, spreading by layers, and having irregular fringed edges. On POTATO a moist, yellowish, spreading growth is formed.

BOUILLON. Grows readily, forming turbidity and aslight covering over the surface.

MILK. It coagulates in two or three days and subsequently is partially peptonized.

ODOR. It has a sour and disagreeable odor.

LITMUS. Acid is produced in milk.

SPORES. Not observed.

STAIN. Readily effected with metheylene blue.

TEMPERATURE. Grows rather slowly at room temperature, but very rapidly at 37 C.

REMARKS. It generally gains access into the milk through the dung, either dropping into the milk or on dried-up particles which float in the air.

BACILLUS INDICUS.

HABITAT. From University of Illinois, Agricultural Department stock.

FORM. Small, narrow, short rods with blunt ends.

MOTILITY. Actively motile.

OXYGEN REG. Aerobic, grows also to the bottom of the stab.

GELATIN. Forms small round grayish colonies which soon liquefy.

GELATIN STAB. Growth all along the stab and liquefies in funnel shape, that is most rapidly from the top.

STREAK CULTURES. On AGAR a white metallic spreading growth which later changes to a faint red. On POTATO the growth is slimy and redder than above, being a brick-red color.

BOUILLON. The medium is very turbid and a slight precipitate is formed.

MILK. Is only coagulated but has no signs of peptonizing.

ODOR. Has very little odor.

LITMUS. Acid is produced.

SPORES. None observed.

STAINS. Stains readily.

TEMPERATURE. Grows at room temperature.

BACILLUS BUTYRICUS.

HABITAT. Milk.

FORM. Long narrow rods three times as long as wide, medium size.

MOTILITY. Actively motile.

OXYGEN REG. Aerobic, grows also to the bottom of the stab.

GELATIN. Small, granular, slightly yellowish, and liquefying rather rapidly.

GELATIN STAB. Grows all along the line of inoculation, liquefies most rapidly from the surface.

STREAK CULTURES. On AGAR it forms a yellowish spreading, slightly wrinkled growth. On POTATO yellowish spreading growth all over the surface.

BOUILLON. Grows very readily turning the medium turbid.

MILK. A very soft coaglum is formed which subsequently is almost completely peptonized.

ODOR. Has a disagreeable putrefactive odor.

LITMUS. Shows a slight acid reaction.

SPORES. Forms oval spores.

STAINS. Reacts readily.

TEMPERATURE. Grows readily at room temperature. Also at 13 to 14 C. but much better at 37 C.

BACILLUS SUBTILIS.

HABITAT. Milk, air, etc.

FORM. Rather large thick rods, about two to three times as long as wide. Often in threads.

MOTILITY. Actively motile.

OXYGEN REG. Aerobic, grows also to the bottom of the stab.

GELATIN. Small, white, granular colonies, spreading in rays from the center. It soon liquefies.

GELATIN STAB. Liquefies along the entire line of inoculation, most rapidly at the top.

STREAK CULTURES. On AGAR it forms a whitish folded pellicle. On POTATO a yellow whitish folded growth covering the greater part of the surface.

BOUILLON. Medium is slightly turbid with a wrinkled growth over the surface.

MILK. Coagulated and almost immediately peptonizes forming a reddish brown liquid.

ODOR. Peculiar sweetish odor.

LITMUS. Considerable acid is produced.

SPORES. Forms large oval spores near the middle of the cell without enlargement.

STAIN. Stains readily.

TEMPERATURE. Grows at room temperature and better at 37 C.

REMARKS. It is commonly called "hay bacillus". It is very common especially in grasses.

BACILLUS PRODIGIOSUS.

HABITAT. Often found in milk, bread, meat, etc.

FORM. Small short rods about two times as long as wide.

MOTILITY. Not motile.

OXMGEN REG. Grows also to the bottom of the stab. Aerobic.

GELATIN. Irregular round colonies, liquefying forming saucer shaped depressions. Red pigment is soon produced.

GELATIN STAB. Growth along the entire line of inoculation, liquefaction most rapid near the top, thus forming a funnel. The liquefied portions turn red.

STREAK CULTURES. On AGAR a dense moist growth is developed which is very deep red. On POTATO the color is darker than on agar.

BOUILLON. The medium is turbid then turns red with a red pellicle.

MILK. Milk turns red then is coagulated without subsequent peptonization.

ODOR. A slight putrefactive odor not very disagreeable.

LITMUS. No acid produced.

SPORES. Not observed.

STAINS. Stains readily.

TEMPERATURE. It grows readily at room temperature.

BACILLUS LACTIS ALBUS.

HABITAT.. Milk, found in-completely sterilized milk.

FORM. Rather large rods three times as long as wide, having blunt ends.

MOTILITY. Motile.

OXYGEN REG. Aerobic. Grows also to the bottom of the stab.

AGAR STAB. Growth along the entire line of inoculation, slightly feathery, most of the growth over the surface.

STREAK CULTURES. On AGAR a white, spreading, glistening growth. On POTATO a small amount of white growth.

BOUILLON. Liquid very turbid and a ring around the surface.

MILK. A soft coagulum is formed which subsequently peptonizes, the peptonized liquid is clear as water.

ODOR. Quite pleasant, very slightly sour.

LITMUS. Slightly acid.

SPORES. Large oval spores are quickly produced.

STAINS. Is easily affected with stains.

TEMPERATURE. Grows at 33 better at 37, very little or not at all at ordinary room temperature.

REMARKS. Was found in condensed milk that had been kept in an incubator for several days.

BACILLUSS ACIDI LACTICI.

HABITAT. Sour milk.

FORM. Short medium sized rods, often in twos and sometimes more.

MOTILITY. Not motile.

OXYGEN REG. Aerobic, grows also to the bottom of the stab.

GELATIN. White colonies spreading with wavy borders. Under the microscope they are yellowish and granular.

GELATIN STAB. Growth all along but more abundant at surface. It does not liquefy.

STREAK CULTURES. On AGAR a thin, white, glistening, wavy spreading growth, On POTATO a shiny white growth is formed.

BOUILLON. Medium slightly turbid with a small precipitate.

MILK. Forms a soft coagulum which is only slightly peptonized. Old cultures will only produce acid but will not coagulate.

ODOR. A peculiar sour odor.

LITMUS. Acid is quickly produced.

SPORES. Not observed.

STAIN. Stains readily.

TEMPERATURE. Grows readily at room temperature.

REMARKS. This is the regular sour milk germ, which causes normal souring of milk and ripening of creams.

MICROCCUS AROMAFACIENS.

HABITAT. Ripe cream.

FORM. Rather small coccus, seldom in twos.

MOTILITY. Not motile.

OXYGEN REG. Aerobic, grows also to the bottom of the stab.

GELATIN. Small, yellowish, granular, regular outlined colonies.

GELATIN STAB. Growth along the entire line. It liquefies most rapidly from the top.

STREAK CULTURE. On AGAR it forms a glistening porcelain, white finely to bed elevated growth.

BOUILLON. Medium becomes turbid.

MILK. Milk is slowly coagulated with but little peptonization.

ODOR. Has a pleasant but not a strong odor.

LITMUS. A small amount of acid is produced.

SPORES. Not observed.

STAIN. Stains readily.

TEMPERATURE. Grows at ordinary room temperature.

REMARKS. This is one of the forms that give aroma to butter and this particular one is furnished by the Duplex Butter Co. as a culture to add to cream to produce good butter.

MICROCCUS AQUATILIS.

HABITAT. In air and milk.

FORM. Very small Micrococcus often in pairs.

MOTILITY. Not motile.

OXYGEN REG. Aerobic, grows also to the bottom of the stab.

GELATIN. White spreading moist colonies.

GELATIN STAB. White, spreading over the surface. There is growth all along the stab, having a kind of feathery appearance. No liquefaction.

STREAK CULTURES. On AGAR white regular growth. On POTATO a thrifty growth the same color as the potato.

BOUILLON. Grows rather slow, turns the medium turbid.

MILK. Does not coagulate or change in color.

ODOR. Has a peculiar but not very pleasant odor.

LITMUS. Not affected.

SPORES. Not observed.

STAIN. Reacts readily with stains.

TEMPERATURE. Grows at ordinary room temperature.

REMARKS. This sample was obtained from the air of the University Dairy.

MICROCOCCUS ACIDI LACTICI LIQUEFACTUS.

HABITAT. Air and milk.

FORM. Very large micrococcus, very often in pairs.

MOTILITY. Not motile.

OXYGEN REG. Aerobic, grows also to the bottom of the stab.

GELATIN. Small white spreading colonies.

GELATIN STAB. Growth along the entire line of inoculation, liquefying from the top.

STREAK CULTURES. On AGAR it forms a white growth which spreads irregularly. On POTATO a grayish thrifty growth is produced.

BOUILLON. It quickly forms a turbid medium.

MILK. Soon coagulates but is not peptonized.

ODOR. Is between sour and putrefactive.

LITMUS. Acid is produced.

SPORES. Not observed.

STAIN. Stains readily with methylene blue.

TEMPERATURE. Grows best in room temperature.

REMARKS. This was found in the air of the University barn.

DIPLOCOCCUS CITREUS CONGLOMERATUS.

HABITAT. Air and milk.

FORM. Medium to large coccus mostly in pairs.

MOTILITY. Not motile.

OXYGEN REG. Aerobic. Grows also to the bottom of the stab.

GELATIN. Small yellow colonies are produced.

GELATIN STAB. Growth all along the stab, liquefies from the top, and leaves the liquified part yellow.

STREAK CULTURES. On AGar yellow, spreading, rather irregular growth. On POTATO a bluish, thrifty growth, gradually turning to a whitish powder.

BOUILLON. Is soon turned turbid.

MILK. Not apparently affected, but a yellow ring is produced at the surface, it later turns acid.

ODOR. Has a peculiar cheesy odor.

LITMUS. Acid is produced.

SPORES. Not observed.

STAIN. Reacts readily with methylene blue.

TEMPERATURE. Grows at room temperature.

DIPLOCOCCUS CITREOUS LIQUEFACIENS.

HABITAT. Air and milk.

FORM. Medium to large coccus, mostly pairs.

MOTILITY. Not motile.

OXYGEN REG. Aerobic.

GELATIN. Colonies round yellowish in the medium, spreading irregularly at the surface.

GELATIN STAB. Liquefies from the top, does not grow into the medium.

STREAK CULTURES. On AGAR a brownish yellow pigment is produced, the growth is not very abundant. On POTATO a yellow thick growth is produced.

BOUILLON. A precipitate and yellow ring are produced.

MILK. A solid coagulum is produced which has a yellow surface. It is not subsequently peptonized.

ODOR. The odor is putrefying.

LITMUS. Acid is produced.

SPORES. Not observed.

STAIN. Reacts readily with methylene.

TEMPERATURE. Grows at room temperature.

REMARKS. Obtained from the air of the University farm.

DIPLOCOCCUS FLAVUS LIQUEFACTUS TARDUS.

HABITAT. Air and milk.

FORM. Small coccus in pairs.

MOTILITY. Not motile.

OXYGEN REG. Aerobic, grows also to the bottom of the stab.

GELATIN. Large yellow spreading colonies.

GELATIN STAB. The growth spreads over the surface but does not liquefy.

STREAK CULTURES. On AGAR it forms a shining, yellow, irregular, spreading growth. It is moist and thrifty at first, which shrinks

later. On POTATO a yellowish juicy growth but not very thrifty.

BOUILLON. Grows well.

MILK. No very great effect except a slight amount of yellow pigment is deposited around the surface of the milk.

ODOR. Has a putrefactive odor,but slight.

LITMUS. No acid is produced.

SPORES. Not observed.

STAIN. Stains readily.

TEMPERATURE. Grows at ordinary room temperature.

BACILLUS NO. I.

HABITAT. Separated from a can of spoiled condensed milk.

FORM. Short thick rods with thick cell.walls. Occurs also in pairs or filaments.

MOTILITY. Not motile.

OXYGEN REG. Aerobic, grows also to the bottom of the stab.

GELATIN. Spherical, irregular, grangular, not spreading, white colonies.

GELATIN STAB. Growth occurs along the entire line of inoculation. No liquefaction.

STREAK CULTURES. On AGAR there is a white growth not spreading. On POTATO there is only a very slight growth, hardly noticeable.

BOUILLON. Is rendered turbid in a short time.

MILK. Rather quickly coagulates the milk, which is subsequently almost completely digested.

ODOR. Has no very disagreeable odor but rather pleasant.

LITMUS. Acid is produced.

SPORES. Not observed.

STAIN. Reacts readily with methylene blue.

TEMPERATURE. Grows at room temperature, but much better at 33 and 37 C.

BACILLUS NO. II.

HABITAT. Separated from a sample of spoiled condensed milk.

FORM. Very small rods with obtuse ends, often found in pairs.

MOTILITY. Actively motile.

OXYGEN REG. Aerobic, grows also to the bottom of the stab.

GELATIN. Small white colonies, liquefying quickly.

GELATIN STAB. Growth all along and liquefaction from the top. A sediment is deposited at the bottom of the liquefaction.

STREAK CULTURES. On AGAR there is formed a white, glistening, spreading growth. On POTATO a dirty white, elevated growth which changes to brown.

BOUILLON. Grows rapidly and forms a precipitate.

MILK. Coagulates the casein readily which is subsequently peptonized leaving an almost clear liquid at the top.

ODOR. It has a very disagreeable putrefying odor.

LITMUS. No acid is produced.

SPORES. Not observed.

STAINS. Quite readily stained with methylene blue.

TEMPERATURE. Grows slowly at room temperature, somewhat faster at 33 and 37 C.

REMARKS. Produces gas, and a viscid stringy growth in all media.

BACILLUS NO. III.

HABITAT. Separated from a sample of spoiled condensed milk.

FORM. Large,short, thick rods having blunt ends.

MOTILITY. Slightly motile.

OXYGEN REG. Aerobic, grows also to the bottom of the stab.

GELATIN. Large, spreading colonies, liquefying and forming a scum over the surface.

GELATIN STAB. Some growth all along but most thrifty and all liquefaction from the top.

STREAK CULTURES. On AGAR a cream colored, quite thrifty, slightly wrinkled growth. On POTATO no growth was obtained.

BOUILLON. The liquid is clear but a precipitate and a pellicle are produced.

MILK. Coagulates and is soon peptonized.

ODOR. Has no strong odor, very slightly putrefactive.

LITMUS. No acid is produced.

SPORES. Large round spores are produced.

STAIN. Stains readily.

TEMPERATURE. Grows at room temperature but more rapidly at 33 anu 37 C.

BACILLUS NO. IV.

HABITAT. Separated from a spoiled sample of condensed milk.

FORM. Short thick rods having rounded ends and often found in twos or more.

MOTILITY. Motile.

OXYGEN REQ. Grows also to the bottom of the stab. Aerobic.

GELATIN. Spherical, very small, granular colonies. Under the high power yellowish, spreading.

GELATIN STAB. White spreading colonies all along the inoculation. No liquefaction.

STREAK CULTURES. On AGAR small amount of white growth. On POTATO the growth is very thin.

BOUILLON. Slightly turbid.

MILK. Milk is coagulated and subsequently peptonized.

ODOR. Has a putrefying odor.

LITMUS. Acid is produced.

SPORES. Not observed.

STAIN. Reacts readily.

TEMPERATURE. Grows at room temperature but better at 37 C.

BACILLUS NO. V.

HABITAT. Separated from a spoiled sample of condensed milk.

FORM. Rather large, long rods and with blunt ends, and often forms filaments.

MOTILITY. Motile.

OXYGEN REQ. Aerobic, grows also to the bottom of the stab.

GELATIN. Very small, white granular colonies.

GELATIN STAB. Very little growth but all along the line of inoculation.

STREAK CULTURES. On AGAR white, spreading, irregular, quite thrifty growth. On POTATO no growth occurred.

BOUILLON. Turns slightly turbid.

MILK. Seems to make the milk more viscid, otherwise no noticeable change.

ODOR. No particular odor is noticed.

LITMUS. A small amount of acid is produced.

SPORES. Large oval spores are produced.

STAIN. Is stained quite readily.

TEMPERATURE. It is very hard to get a growth at rrom temperature, but grows at 33 and still better at 37 C.

BACILLUS NO. VI.

HABITAT. Separated from a sample of spoiled condensed milk.

FORM. Rather large, short, thick rods having blunt ends and often forming long chains.

MOTILITY. Motile.

OXYGEN REG. Aerobic, grows also to the bottom of the stab.

GELATIN. White spreading colonies. Under the microscope they are yellowish, granular even spreading, the liquefaction first forms little pits.

GELATIN STAB. Growth all along the stab with liquefaction most rapid at the surface.

STREAK CULTURES. On AGAR a grayish spreading growth which has a decided metallic luster. On POTATO an abundance of grayish growth which is very even and smooth.

BOUILLON. Forms a precipitate and a pellicle over the surface, the liquid remaining quite clear.

MILK. Is coagulated and then starts peptonizing until the coagulum has almost completely disappeared.

ODOR. Has a mixed, sour and putrefying odor.

LITMUS. Acid is produced.

SPORES. Large oval spores are formed quite readily.

STAINS. Stains readily.

TEMPERATURE. Grows at room temperature but better at 37 C.

BACILLUS NO. VII.

HABITAT. Separated from a sample of spoiled condensed milk.

FORM. Large rods with blunt ends, being five times as long as wide.

MOTILITY. Not motile.

OXYGEN REG. Aerobic, grows also to the bottom of the stab.

GELATIN. Small white granular colonies.

GELATIN STAB. Grows all along the stab without liquefying gelatin.

STREAK CULTURES. On AGAR an abundance of white spreading growth, which has a slight metallic luster. On POTATO a small amount of white growth.

BOUILLON. Grows slowly and renders it slightly turbid.

MILK. Slightly thicker, not otherwise affected.

ODOR. No odor.

LITMUS. No acid produced.

SPORES. Oval spores are formed.

STAIN. Stains readily.

TEMPERATURE. Grows slowly at room temperature, much better at 37 C.

BACILLUS NO. VIII.

HABITAT. Separated from imperfectly sterilized gelatin.

FORM. Medium sized, long slender rods with rounded edges.

MOTILITY. Actively motile.

OXYGEN REG. Aerobic.

GELATIN. Small, white, glistening colonies, with fringed edges.

GELATIN STAB. Grows only at the surface and does not liquefy.

STREAK CULTURES. On AGAR a thrifty, white, glistening growth which later changes to a cream color. On POTATO very little growth, a moistness appearing at the place of inoculation.

BOUILLON. The liquid remains clear but it forms a pellicle over the surface.

MILK. Shows no signs of growth in milk but the presence of bacteria can be noticed under the microscope.

ODOR. Very little or no odor is produced.

LITMUS. Not affected.

SPORES. Rather large spherical spores are found.

TEMPERATURE. Grows very slowly at room temperature, somewhat faster at 37 C.

BACILLUS NO. IX.

HABITAT. From the air of the University of Illinois barn.

FORM. Quite large rods almost two times as long as wide. They sometimes form short chains.

MOTILITY. Slightly motile.

OXMGEN REG. Aerobic, grows also to the bottom of the stab.

GELATIN. White, irregular, spreading colonies.

GELATIN STAB. Grows along the entire length of the stab, liquefying in a funnel shape.

STREAK CULTURES. On AGAR it forms a smooth glistening surface which later dries and wrinkles. On POTATO, a thrifty growth about the same color as the potato.

BOUILLON. Forms a precipitate and a pellicle but leaves the liquid turbid.

MILK. Coagulates and peptonizes rapidly and almost completely, leaving the liquid a kind of a yellowish color.

ODOR. A putrefying disagreeable odor.

LITMUS. Acid is produced.

SPORES. Oval spores are produced.

STAIN. Reacts readily with methylene blue.

TEMPERATURE. Grows rapidly at room temperature.

BACILLUS NO. X.

HABITAT. Sour milk.

FORM. Small rods with round ends being about two times as long as wide.

MOTILITY. Actively motile.

OXYGEN REQ. Aerobic, grows also to the bottom of the stab.

GELATIN. Small granular colonies liquefying very rapidly.

GELATIN STAB. Grows all along the line of inoculation, liquefies in a funnel shape.

STREAK CULTURES. On AGAR it forms a white, spreading, glistening, growth. On POTATO the growth is grayish white, covering the entire potato.

BOUILLON. Liquid is rendered turbid.

MILK. Forms a hard coagulum which subsequently peptonizes, leaving a clear watery liquid.

ODOR. Has a peculiar sour odor.

LITMUS. Acid is produced.

SPORES. Not observed.

TEMPERATURE. Grows quite rapidly at room temperature.

BACILLUS NO. XI.

HABITAT. Separated from the foremilk of a cow.

FORM. Medium sized, short, thick rods, with blunt ends, being about two times as large as wide. It sometimes forms very short chains.

MOTILITY. Not motile.

OXYGEN REQ. Aerobic, grows also to the bottom of the stab.

GELATIN. Small, whitish, regular, granular colonies.

GELATIN STAB. Growth all along but more abundant at the surface. Not liquifying.

STREAK CULTURES. On AGAR the growth is white, spreading, glistening, with a slight metallic luster. On POTATO a thrifty grayish growth.

BOUILLON. Grows slowly but renders medium turbid.

MILK. After about a week there is an appearance of coagulation, the coagulum is very soft.

ODOR. A rather pleasant odor.

LITMUS. Acid is produced.

SPORES. Not observed.

TEMPERATURE. Grows rather slowly at room temperature, better at 33 C

BACILLUS NO. XII.

HABITAT. Separated from imperfect sterilized agar.

FORM. Short thick rods often in short chains.

MOTILITY. Slowly motile.

OXYGEN REG. Aerobic.

GELATIN. Spherical, granular, yellowish, regular outlined colonies.

GELATIN STAB. Growth only at the surface, liquefying.

STREAK CULTURES. On AGAR white spreading, glistening growth. On POTATO a large amount of elevated growth, the same color as the potato.

BOUILLON. The medium becomes bery turbid.

MILK. Not affected.

ODOR. No odor is noticed in milk but in gelatin a very disagreeable rotten odor.

LITMUS. No acid is produced.

SPORES. Not observed.

STAIN. Stains with gentian violet.

TEMPERATURE. Grows quite rapidly at room temperature.

BACILLUS NO. XIV.

HABITAT. Separated from sewage water.

FORM. Rather large long rods with blunt ends.

MOTILITY. Slowly motile.

OXYGEN REG. Aerobic, grows also to the bottom of the stab.

GELATIN. Yellowish, granular, colonies spreading in a thin film over the surface.

GELATIN SLAB. Grows all along the inoculation but most rapidly at the surface. It does not liquefy.

STREAK CULTURES. On AGAR the growth is yellowish, very thrifty, spreading slightly with frizzy edges. On POTATO a slight amount

of white growth at the place of inoculation.

BOUILLON. Forms a precipitate leaving the liquid turbid.

MILK. Is not coagulated but forms a yellow precipitate.

ODOR. Has a slight putrefactive odor.

LITMUS. No acid is produced.

SPORES. Oval spores are formed quite readily.

STAIN. Reacts with the ordinary stains.

TEMPERATURE. Grows rapidly at room temperature.

BACILLUS NO. XV.

HABITAT. Found in perfectly sterilized potato.

FORM. Large rods with rounded ends, being about two times as long as wide.

MOTILITY. Slowly motile.

OXYGEN REG. Aerobic.

GELATIN. The colonies at first spherical then spreading irregularly.

GELATIN STAB. Growth only at the surface, it liquefies and forms a scum, also a precipitate at the bottom of the liquefied part.

STREAK CULTURES. On AGAR a spreading, white, white, wrinkled growth.

On POTATO a large amount of wrinkled growth pinkish in color.

BOUILLON. Grows rather slow, forming a pellicle and leaving the liquid clear.

MILK. Is coagulated rather quickly and subsequently completely peptonized. The liquid remaining is a dirty slightly yellowish color.

ODOR. Is very bad putrefying.

LITMUS. No acid is produced.

SPORES. Oval spores are produced.

TEMPERATURE. Grows readily at room temperature.

BACILLUS NO. XVII.

HABITAT. Separated from the stripping of cow's milk.

FORM. Rather small slender rods about three times as long as wide. It often forms chains.

MOTILITY. Slowly motile.

OXYGEN REG. Aerobic, grows also to the bottom of the stab.

GELATIN. Yellowish spreading colonies, with irregular outlines.

GELATIN STAB. Most of the growth is at the surface, but some growth the entire length of the stab. It liquefies very slowly.

STREAK CULTURES. On AGAR a yellowish spreading growth. On POTATO a small amount of growth the same color as the potato.

BOUILLON. The medium becomes very turbid and a slight precipitate is formed.

MILK. No coagulation occurs but the milk becomes slightly yellow.

ODOR. A sweetish disagreeable odor is produced.

LITMUS. No acid is produced.

SPORES. Not observed.

STAIN. Reacts readily with methylene blue.

TEMPERATURE. Grows readily at room temperature.

BACILLUS NO. XVII.

HABITAT. Obtained from the air of the University of Illinois barn.

FORM. Very small rods about four times as long as wide.

MOTILITY. Actively motile.

OXYGEN REG. Aerobic.

GELATIN. Small, white, spherical colonies.

GELATIN STAB. Grows only at the surface and liquefies slowly.

STREAK CULTURES. On AGAR yellowish gray, quite thrifty; spreading growth. On POTATO an elevated dirty yellow shining growth.

BOUILLON. Produces a white pellicle leaving the liquid slightly turbid.

MILK. Is quickly coagulated and later almost completely dissolves the coagulum, leaving a dirty yellow liquid.

ODOR. A very bad putrefying odor.

LITMUS. Acid is produced.

SPORES. Not observed.

STAIN. Stains with methylene blue.

TEMPERATURE. Grows at room temperature.

BACILLUS NO. XVIII.

HABITAT. Separated from imperfectly sterilized agar.

FORM. Slender but very small rods with blunt ends, found to form filaments..

MOTILITY. Motile.

OXYGEN REG. Aerobic.

GELATIN. Small, yellowish, granular, irregular, spreading. The plate having a very bad putrefying moldy odor.

GELATIN STAB. Grows only at the surface and liquefies very slowly.

STREAK CULTURES. On AGAR a cream colored, irregular, spreading growth. On POTATO a yellowish brown, moist, thrifty growth.

BOUILLON. Grows rapidly and renders the medium turbid.

MILK. Is not coagulated but the color is changed to a slight yellowish.

ODOR. The odor is bad putrefying similar to that of the plate.

LITMUS. A slight amount of acid is produced.

SPORES. Not observed.

TEMPERATURE. Grows very readily at room temperature.

BACILLUS NO. XIX.

HABITAT. Separated from the foremilk of a cow.

FORM. Short, thick, medium sized rods with blunt ends and having very thick cell walls.

MOTILITY. Not motile.

OXYGEN REQ. Aerobic, grows also to the bottom of the stab.

GELATIN. White, spherical colonies. Those at the surface spread quite rapidly.

GELATIN STAB. Growth all along the stab, spreading over the surface and slowly liquefying.

STREAK CULTURES. On AGAR a white thrifty growth. On POTATO a yellowish gray, shining, thrifty growth.

BOUILLON. Is rendered turbid and a precipitate is formed.

MILK is soon coagulated and subsequently peptonized.

ODOR. A pleasant sour odor, not very strong.

LITMUS. A considerable acid is produced.

SPORES. Not observed.

STAIN. Reacts with methylene blue.

TEMPERATURE. Grows readily at room temperature.

REMARK. The rods are much larger on potato than on other media, and sometimes it is found in twos or pairs.

MICROCOCCUS. NO. I.

HABITAT. Separated from incompletely sterilized milk.

FORM. Very small spheres, seldom in twos.

MOTILITY. Not motile.

OXYGEN REG. Aerobic.

GELATIN. The growth on gelatin was not observed because it could not be grown except in incubator.

AGAR PLATE. Small, round, spreading colonies are produced.

STREAK CULTURES. On AGAR a thin white spreading growth irregular in outline. On POTATO quite thrifty, slightly elevated, grayish growth.

BOUILLON. Grows quite rapidly and the medium is rendered turbid.

MILK. Is soon coagulated and subsequently peptonized. The coagulum is rather hard and tough.

ODOR. Very little odor if any is produced.

LITMUS. Acid is produced.

SPORES. Not observed.

STAIN. Reacts with methylene blue.

TEMPERATURE. Does not grow in room temperature but very rapidly at 35 or 37°C.

MICROCOCCUS NO. II.

HABITAT. Separated from a spoiled sample of condensed milk.

FORM. Small spheres, often in twos and fours.

MOTILITY. Not motile.

OXYGEN REG. Aerobic. Grows also to the bottom of the stab.

GELATIN. Small white colonies, under the microscope they appear as yellowish, granular, regular outlines.

GELATIN STAB. Grows along the entire length of the stab, no liquefaction takes place.

STREAK CULTURES. On AGAR a very thrifty white growth. On POTATO a small amount of grayish colored growth.

BOUILLON. Produces a slight precipitate, and renders the medium slightly turbid.

MILK. Apparently not affected.

ODOR. An odor similar to that of sour milk is noticed, except that it is fainter.

LITMUS. Acid is produced.

SPORES. Not observed.

STAINS. Reacts readily with methylene blue.

TEMPERATURE. Grows at room temperature and at 37 C.

DIPLOCOCCUS NO. I.

HABITAT. Separated from the foremilk of a cow.

FORM. Medium sized spheres mostly in twos.

MOTILITY. Not motile.

OXYGEN REG. Aerobic, grows also to the bottom of the stab.

GELATIN. Small, spherical, granular, irregular colonies.

GELATIN STAB. There was a yellowish growth along the entire line of inoculation, most growth at the surface, but no liquefaction.

STREAK CULTURES. On AGAR a considerable amount of pale yellowish growth. On POTATO an abundance of white powdery elevated growth.

BOUILLON. Renders it turbid.

MILK. Coagulates and later the coagulum is digested.

ODOR. Has a pleasant sour odor.

LITMUS. Acid is produced.

SPORES. Not observed

TEMPERATURE. Grows rather slowly at room temperature.

DIPLOCOCCUS NO. II.

HABITAT. Obtained from the air of the University barn.

FORM. Medium to large spheres mostly in twos.

MOTILITY. Not motile.

OXYGEN REG. Aerobic. Grows also to the bottom of the stab.

GELATIN. Small, yellowish, spherical colonies, liquefying in saucer shaped pits.

GELATIN STAB. Growth along the entire line of inoculation, liquefying in a funnel shape.

STREAK CULTURES. On AGAR a yellowish, glistening, quite thrifty growth. On POTATO a small amount of yellowish growth.

BOUILLON. Is rendered very turbid.

MILK. Milk is soon coagulated producing a rather soft coagulum. It is afterwards peptonized, producing a slightly yellowish liquid, and not very clear.

ODOR. A peculiar musty putrefying odor is produced.

SPORES. Not observed.

TEMPERATURE. Grows very rapidly at room temperature.

DIPLOCOCCUS NO. III.

HABITAT. Obtained from the air of the laboratory.

FORM. Rather large spheres mostly in twos but sometimes in fours.

MOTILITY. Not motile.

OXYGEN REQ. Aerobic, grows also to the bottom of the stab.

GELATIN. Small white colonies.Under the microscope yellowish, granular and spreading evenly.

GELATIN STAB. Growth along the entire line and not liquefying.

STREAK CULTURES. On AGAR a white irregular, spreading growth. On POTATO a small amount of grayish growthy changing to a white powder.

BOUILLON. A small precipitate is produced and the medium rendered only slightly turbid.

MILK . The milk is apparently not changed.

ODOR. Has a peculiar putrefying odor, appearing to be somewhat musty.

LITMUS. Very slightly acid.

STAIN. Reacts rather slowly with methylene blue.

SPORES. Not observed.

TEMPERATURE. Grows rather slowly.

Turning now to the chemistry of the subject we know that nearly every species of bacteria produces some kind of by-product. Quite often poisons are separated from milk and these are in most cases caused by the action of bacteria. The poisons often known as toxins, are ptomains. A ptomain, according to Novy, may be defined as an "organic chemical compound, basic in character and formed by the action of bacteria on nitrogenous matter'. Ptomains consist of carbon, hydrogen, nitrogen and some oxygen. They are alkaline substances, which, when neutralized by acids yield crystallizable salts. Some of the ptomains are highly poisonous, this is however not an essential property because others are wholly inert. Brienger restricts the term ptomain to the non-poisonous products, and calls the poisonous ones toxins.

The presence of poison producing bacteria in milk has often cauned a great amount of trouble. They occur in all our food as well as in milk. Many cases have been reported where toxin was present in milk and caused serious illness and often death, to all the members of a family or many of the patrons of a dairyman. The same trouble has been reported from ice cream. The milk is seemingly not affected when consumed and nothing wrong is suspected until a few hours afterwards. The poison is generally present in such small quantities that it can not be detected by a chemical analysis: but remembering that the minutest particle of a grain is enough to kill a man we do not wonder why such disastrous results follow the use of such food.

Most of the disease producing forms do their damage to the body by the production of poison. They do not produce the poison in the milk but when most active in their growth within the body.

When the production of poisons reaches a certain point it will prove detrimental to the bacteria themselves and if the patient is able to resist this point the disease will not be fatal.

This leads us to the thought of immunity. A person once having had a disease is not liable to take it again for a long time. The disease produces in the system what is known as an anti-toxin. This anti-toxin is produced even when a person has the disease in a very mild form. In this way vaccines are made use of; a mild form of a disease is inoculated and a patient is rendered immune. The inoculation of anti-toxins has also been made use of in the treatment of diphtheria. It must be remembered that each species of bacteria has its own anti-toxin. Certainly we do not expect a man to be immune to all diseases just because he has had one disease.

Most of the common forms found in milk do not produce toxins but they do act upon the nitrogenous materials. The action of most bacteria upon butter fat is very slight. The per cent in milk has been found to remain constant whatever the kind of bacteria acting upon it. The amount of ash also varies very little because of bacteria need but the slightest quantity for their development. The sugar, as a rule, is greatly affected, but differing with different forms. In the decomposition there may be a number of products formed but among the more common are lactic acid, butyric acid, carbonic acid, water, etc. It is not necessary to go into full detail as to the reactions that take place in the decomposition of milk sugar.

The nitrogenous materials, that is the casein and albumen are also greatly changed. Nitrogenous materials are absolutely essential for the life of bacteria and these being the only nitrogen-containing compounds in milk are consequently greatly attacked

by bacteria. As to what extent these nitrogenous products are affected and what is formed on their decomposition very little is known. W. Kühne in the Journal of the Chemical Society, 1893, p. 232 reported that in Koch's work it was found that Bacillus of tuberculosis produced proto-albumose, and deutro-albumose but no peptones. Hay bacillus when acting on milk also produce peptones. Surely these nitrogenous materials, the most expensive of our food constituents are important enough to be looked after. It was found on the analysis of a sample of spoiled milk that there was only a trace of casein left in the milk, showing that nearly all of it has been changed to something else.

It was thought well worth the time to find out the effect of different bacteria upon milk, but more especially upon the nitrogenous materials in the milk, and to determining what is formed and in what amounts. With this end in view several samples of milk were sterilized and a complete analysis was made of one sample; the other samples were inoculated with different species. For this purpose skim milk was used for several reasons, 1st it is known that bacteria do not affect the butter fat to any great extent, and 2nd the milk is easier to handle when there is no cream present.

About 500 c.c. of skim milk was placed in a flask and sterilized in an autoclave at ten pounds pressure. This was allowed to stand a few days to see if it was sterile. It was then inoculated with a single culture. This was then allowed to act upon the milk for about a month. These flasks inoculated with B. subtilis, B. mesentericus vulgatus, B. butyricus, and B. coli communis were kept in the incubator to allow the most possible growth in that time. The other forms were kept at room temperature and

in a rather dark place. After they had worked for about a month a complete chemical analysis was made of each.

The blank sample was first analyzed for the specific gravity, total solids, ash, proteids, sugar, and acidity; casein was also tested for and subtracted from total proteids to determine the amount of albumen. The affected sample was analyzed as the above with the addition of a test for rennet. The casein test was abandoned after the first few analyses, for it was found that only the undigested or undissolved portions would give a test for casein. Rennet was tested for according to the method of Conn of storr's station. The solution was run through a Bergerfeld filter, and part of the sterile solution was transferred by means of a sterile pipette to some sterile milk and some to beef broth as a check for sterility. These were then placed in an incubator; if coagulation occurred and no growth in the broth, it showed that the coagulation was caused by rennet and not by the action of bacteria. The sugar was determined by means of the polariscope. The acidity was determined by neutralizing with one-tenth normal sodium hy dioxide with phenolphthalein as an in dicator. Casein was determined by saturating the milk with magnesium sulphate, filtering and determining the amount of proteids according to the Kyeldhal method. The other determinations were according to the methods given in my thesis for the degree of B.S. that is, according to the method of analysis adopted by the Association of Official Agricultural Chemists.

Considerable difficulty was found in sampling the milk. The small lumps of casein could not be dissolved by the addition of acids or alkalis, so it was decided to filter them out and call them undigested casein. If it was present in large quantities it

was impossible to dry this to constant weight so it was determined to get at this by the difference between the amount of total nitrogen in the original and the amount in the filtrate.

For the separation of the nitrogenous compounds a method was obtained from the brief diagram given in A.H.Allen's Commercial Organic Analysis, vol. IV second edition p. 26. It was however found advisable to make a few changes, and from the nature of the material it was found necessary to make all the tests. The method employed was as follows:

100 c.c. of the filtered material is placed in a beaker and exactly neutralized to phenolphthalein. The precipitate is filtered off and the amount of nitrogen determined, by inserting precipitate filter paper and all into a digestion flask and then by proceeding as usual. This gives the amount of ac id albumen by using the factor 6.25.

The filtrate was divided into two equal portions. Part A was slightly but distinctly acidulated with acetic acid, then boiled for a few minutes and filtered. The precipitate represents the amount of albumens or globulins. The filtrate is then saturated with zinc sulphate. The zinc sulphate must be ammonia free and it must be finely powdered in order to insure saturation. It is then filtered and the precipitate washed with a saturated solution of zinc sulphate The washing is doneon the filter. This gives the amount of deutro-albumose. The filtrate contains in solution the peptone which is determined by running one-fourth or one-tenth according to amount contained, by the Kjeldahl method.

Part B.of the filtrate was first saturated with magnesium sulphate. The same precautions were necessary as with the zinc

sulphate in the other case, that is to have it ammonia free and also to have it finely powdered to insure saturation. It is even harder to get a saturated solution of this than with the zinc sulphate. The precipitate was washed with a saturated solution of magnesium sulphate and the precipitate gave the amount of caseinogen. The filtrate was treated for deutro-albumose, with zinc sulphate as above described, and the filtrate from this contained peptone.

In every case to determine the per cent the precipitate paper and all was put into a Kyeldhal digestion flask and the amount of nitrogen determined. This was multiplied by the factor 6.22 and the amount of material was obtained from which the per cent was easily calculated. The results from the different analyses were as follows:-

RESULTS OF ANALYSES.

These analyses represent the milk after the solids or coagulated portions are filtered off.

No. of Sample	Treated with	Specific gravity	Acidity	Sugar %	Total solids %	Ash %	Protein %	Casein %
480	blank	1.0385	.192	4.2	9.14	.77	3.17	2.92
481	B. subtilis	1.0415	.998	.844	8.33	.85	3.27	.23
486	M. Aromifaciens	1.0233	.328	3.33	5.86	.99	1.076	
530	2nd. blank	1.0355	.172	4.3	8.92	.76	2.89	2.72
533	B. butyricus	1.0362	1.001	1.72	8.17	.73	2.41	
532	B. mesentericus vulgatus	1.0315	.646	3.196	7.13	.68	1.736	
534	B. cyanogenus	1.0312	.165	2.664	7.525	.64	2.43	
531	B. subtilis	1.0355	1.159	1.909	7.981	.77	2.667	
564	3rd blank	1.0340	.154	4.570	8.89	.79	2.824	2.547
565	B. coli communis	1.0305	.369	4.57	6.172	.65	.350	
566	B. acidi lactici	1.0295	.602	3.95	6.113	.70	.509	
585	B. coli communis	1.0295	.427	4.89	6.446	.67	.32	

No. of Sample	Undigested casein* %	acid-albumin %	albumins + globulins %	Albumose %	Peptone %	Caseinogen %	Deutro-albumose %	Peptone %	Rennet
481	.204	.13	.053	.114	2.94	.06	.110	2.97	present
486	2.59	.039	.049	.333	.567	.201	.183	.547	not present
533	.47	.228	.037	.179	1.935	.113	.160	1.932	present
532	1.71	.154	.053	.489	.937	.189	.196	1.119	present
534	.46	.105	.177	.518	1.682	.272	—	1.457	present
531	.159	.118	none	.183	2.257	.078	.259	2.127	present
565	2.474	.101	.017	.119	.172	.068	.062	.131	not present
566	2.315	.129	none	.182	.229	.077	.123	.236	not present

* Casein and other matters seperated by the action of the fermentation.

The samples marked blank represent the test of the milk before inoculation. After each of these are placed analyses of milk inoculated from that particular batch. The analysis of No. 585 was on a fourth batch, which was not analyzed before inoculation.

The specific gravity remains quite constant after inoculation tending to show that the bacteria do not greatly affect this property of the fluid. The acidity and sugar vary together, the sugar decreasing in about the same proportion as the acid increases. This did however not prove true in the case of B. cyanogenus with which the sugar greatly decreased and the acid also slightly decreased. In this case the sugar must have changed to something else than acid. B. coli communis often employed in the process of rendering media sugar free previous to its use for the indol test, has not affected the sugar very much if any at all. It was for this reason the analysis of No. 585 was made. This analysis seems to verify the results obtained in No. 565.

In almost every case the per cent of total solids is lower after the action of bacteria, this is not only true as shown in the column marked total solids, but also when adding to this the per cent of undigested casein, which was filtered off before the determination of total solids. The ash is not materially affected.

It appears from the second part of the table that the per cents of dutro-albumose and peptone are about the same in the two parts of the same sample, the difference being so slight that it might be due to error in chemical analysis. It is not known whether these forms would go farther in the production of peptones if the time were extended. It is very probable that they would in some cases. Novy in a test for poisons in milk caused by bacteria, was

in many cases only able to detect the poisons by letting the milk stand for several months. In other cases the peptones would probably not be increased for in the second analysis of milk treated with B. subtilis and exposed about twice as long as the other case no greater per cent of peptones was found. It is indeed slightly lower but the per cent of protein was lower in the original.

Owing to the lateness at which this part of the work was commenced further analyses were impossible and for this reason no definite conclusions can be drawn but it is evident that there is a great deal of difference in the actions of these bacteria. No two are alike in their action upon milk. They do not only differ in their decomposition of proteins but also in their action upon sugar and in the formation of acid. It seems to the writer that this opens a new field of investigation which should prove very interesting and valuable, if not from the practical side, certainly from the standpoint of the scientist.